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# Detecting and understanding combinatorial mutation patterns responsible for HIV drug resistance

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**We propose a systematic approach for a better understanding of how HIV viruses employ various combinations of mutations to resist drug treatments, which is critical to developing new drugs and optimizing the use of existing drugs. By probabilistically modeling mutations in the HIV-1 protease or reverse transcriptase (RT) isolated from drug-treated patients, we present a statistical procedure that first detects mutation combinations associated with drug resistance and then infers detailed interaction structures of these mutations. The molecular basis of our statistical predictions is further studied by using molecular dynamics simulations and free energy calculations. We have demonstrated the usefulness of this systematic procedure on three HIV drugs, (Indinavir, Zidovudine, and Nevirapine), discovered unique interaction features between viral mutations induced by these drugs, and revealed the structural basis of such interactions.**

Bayesian model selection | free energy calculation | Markov chain Monte Carlo | molecular dynamics | mutation interactions

**H**IV drug-resistance, which is caused by mutations of viral proteins that disrupt the drugs' binding but do not affect the viral survival, is a major hurdle that hinders a successful treatment of AIDS (1, 2). Due to the high rate and low fidelity of HIV replication, resistant strains quickly become dominant in a viral population under the selection pressure of a drug. By sequencing viral strains in the treated-patient isolates, genotypic data have been accumulated for the drugs targeting two viral enzymes, protease and reverse transcriptase, that are essential to the virus's replication. Because each mutation of the viral protein is not equally important for drug resistance, the observed, complicated mutation patterns are difficult to interpret (3, 4) and are limited in helping physicians design the best therapeutic regimen for a patient (5) (Fig. 1A).

In past decades, many statistical learning methods (3, 4, 67–8) have been employed to help predict phenotypes from genotypes. There are also rule-based systems that infer drug-resistance levels from sequence information such as the Stanford University HIV Drug Resistance Database (Stanford HIVdb). However, these methods provide little insight on the genetic and molecular basis of drug resistance and often give inconsistent results when analyzing the same input mutation data (4, 6).

In the present study, we investigated the problem of mutation interactions of the HIV induced by a certain drug treatment. Using a unique probabilistic model, we first detect resistant mutation combinations (9) and infer the interaction dependence structure of these combinations. Then, we use molecular dynamics (MD) simulations to reveal the molecular basis of how these mutations interact with each other to interfere with the drugs' binding. We have shown that our procedure is applicable to different antiretroviral drugs treating different types of HIV infection by analyzing the sequence mutations induced by three different drug treatments: a protease inhibitor (indinavir), a nucleoside analog reverse-transcriptase inhibitor (zidovudine), and a nonnucleoside reverse-transcriptase inhibitor (nevirapine). We have rediscovered the majority of known resistant mutations to

the three drugs (10) and uncovered several interacting structures for these mutations. Particularly, for protease we have discovered a conditional independence structure among the mutations M46I, I54V, and V82A that is consistent with several previous experimental results (3, 5, 6, 111213–14) but has not been documented in the literature. Our MD simulations and free energy analyses have further confirmed and provided the molecular basis and implication of this conditional independence.

## Results

**Analytical Pipeline for Studying HIV Mutation Data.** We first design a Bayesian variable partition (BVP) model, a generalization of the “Bayesian epistasis association mapping” (BEAM) model in Zhang and Liu (9), to select mutations that are associated with drug resistance. Next, we design a recursive model selection (RMS) procedure that recursively partitions a set of mutation positions into three subsets so that the three sets of variables either follow a chain-dependence structure, or a “V” structure (see *Methods* section) to infer the dependence structure among the interacting mutation positions found by the BVP model. Finally, we illustrate the molecular basis of the mutation patterns predicted by BVP and RMS by using molecular dynamics simulations and inhibitor-residue free energy decomposition analyses.

**Complex Interaction Patterns for Drug Resistance of Indinavir.** The data contain 949 HIV-1 (type B) protease sequences from indinavir-treated patients (indinavir is the only PI in their therapy) and 4,146 sequences (HIV-1 type B) from untreated patients. HIV-1 protease has 99 amino acids and each position has mutations in the dataset. Any combination of mutations among these 99 positions may be related to the virus' drug resistance capability. Our goal is to find those positions that are either independently or interactively associated with the indinavir treatment.

Fig 1 shows the posterior probabilities for each marker to be associated interactively with the indinavir treatment based on the BVP model under two different prior distributions. We can see that the results are insensitive to the priors. Nine out of the 10 positions with high posterior probabilities of interaction (i.e., 10, 24, 32, 46, 54, 71, 73, 82, and 90) are on the drug resistance mutation list (5) updated in spring 2008 (Fig S1). The only one not on the list is position 47, which is well-known to be associated with indinavir drug resistance when combined with position 32 (3). We have found 17 mutation patterns (out of a total of 20<sup>99</sup> possibilities) that are associated with indinavir treat-

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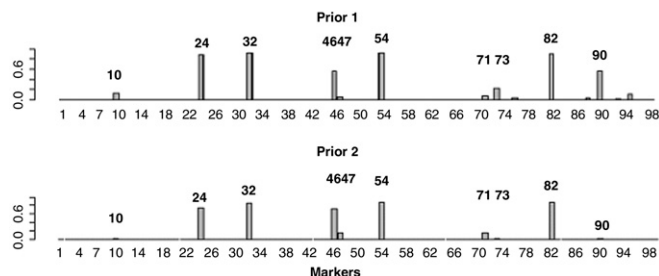
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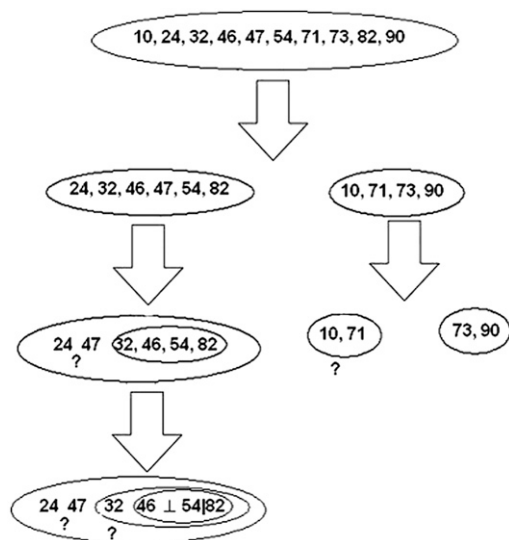


**Fig. 1.** The posterior probabilities for each mutation to be associated interactively with indinavir treatment. The *Upper* shows the posterior probabilities using prior one, which assumes that it is equally likely (1/3) for a mutation to be unassociated, individually associated, and interactively associated with the drug treatment. The *Lower* shows the posterior probabilities using a more stringent prior (prior two) assuming that only two markers are expected to be associated with the drug, either individually or interactively.

ment with a posterior probability  $>0.0001$  (this cutoff is much higher than the equally likely probability  $1/20^{99}$ ). [Table S1](#) in [SI Text](#) tabulates these patterns and their respective posterior probabilities. Phenotypic data from Stanford HIVdb provides confirming evidence for the configurations of the top interaction pattern {24, 32, 46, 54, 82} ([SI Text](#)). Many of these mutations are well-known for their drug resistance effects. For example, it is known that the mutations of V82A/F/T or L90M are necessary but not sufficient for measurable resistance to indinavir (11).

#### Dependence Structure of Interaction for Drug Resistance of Indinavir.

We applied the RMS procedure to infer the detailed dependence structure among the interacting positions 10, 24, 32, 46, 47, 54, 71, 73, 82, and 90 (Fig. 2). Two marginally independent interaction groups were found with high confidence: one is composed mainly of 46, 54, and 82; and the other of 73 and 90 (more details are given in [SI Text](#)). Interestingly, we found a strong conditional independence structure in group {46, 52, 82}. Given the amino acid at position 82, mutations at 46 and 54 are mutually independent. The data did not provide strong enough information regarding the structures for other variables (mutations) in this group, for example, 24, 32, and 47. For the second group, 73 and 90 strongly interact with each other.



**Fig. 2.** Detection of a detailed mutation interaction structure for resisting indinavir. Positions 46 and 54 are conditionally independent given position 82, denoted as  $46 \perp 54 | 82$ . The ? indicates where we are not able to confidently infer the dependence structure ([SI Text](#)).

In the study of Zhang et al. (13), a rebound in virus levels in plasma following the initial sharp decline at the beginning of indinavir therapy was found to be associated with a sequential acquisition of mutations at the protease positions of  $46 \rightarrow 82 \rightarrow 54$ . We further searched the Stanford HIVdb, and found that 112 patients from the treated group (HIV-1, main group, and subtype B) had indinavir as their only PI in their therapy and also have detailed mutation records (more than one complete protease sequences) during the course of therapy. Among them 53.6% (60 patients) have at least one of the mutations at positions 46, 54, and 82. We observed no patient with the single mutation V82I, with the single mutation at 54, or with the double mutations at 46 and 54. Among the 21 patients who have all three mutations, only six of them have detailed mutation records such that we can tell the exact order of sequential acquisition of these three mutations. Four out of the six have the order  $46 \rightarrow 82 \rightarrow 54$ , one has the order  $82 \rightarrow 46 \rightarrow 54$ , and another has the order  $82 \rightarrow 54 \rightarrow 46$ . Whereas all these observed orders are consistent with our inferred conditional independence structure, the non-observed orders,  $46 \rightarrow 54 \rightarrow 82$  and  $54 \rightarrow 46 \rightarrow 82$ , are not. This suggests that the conditional independence is a direct consequence of sequential acquisition of the three mutations.

#### Molecular Basis of Interacting Mutations Revealed by MD Simulations and Free Energy Calculations.

To further investigate the molecular implication of the mutation interactions within the {46, 54, 82} group, and within the {73, 90} group, we conducted MD simulations to analyze the binding free energies of the protease/indinavir complexes ([SI Text](#)). The free energy decomposition analyses for the wild-type and ten mutant proteases (Table 1) show that the drug resistant mutations primarily affect the van der Waals interactions between indinavir and the protease. Most of the mutations in the {46, 54, 82} group show positive relative binding free energies, that is, decrease of indinavir's binding affinity.

Among the three single mutations, two of them (M46I and V82A) substantially increase the indinavir binding free energies ( $-77.30 \pm 0.45$  and  $-75.67 \pm 1.50$ ), whereas I54V does not impair the binding. This result is consistent with our observation in the Stanford HIVdb. Among the 112 patients who have more than one mutation record during their indinavir therapy, 10 have the single mutation at 46, 10 have the single mutation at 82, and zero have the single mutation at 54.

Among the double mutations, M46I/V82A and I54V/V82A severely impair the binding of indinavir whereas M46I/I54V does not significantly weaken the binding of indinavir. Incidentally, among the 112 patients in the Stanford HIVdb, 11 have double mutations at positions 46 and 82, eight at positions 54 and 82, and zero at positions 46 and 54. It appears that 46 and 54 cannot interact to resist to indinavir without the mutation at 82. The observations that double mutations M46I/V82A and I54V/V82A are the two strongest resistant mutants may have important implications for improving the potency of indinavir to combat resistance. If we can decrease the interaction between V82 and a derivative of indinavir without affecting the total binding affinity of the inhibitor, the resistant effects of 46 and 54 will be reduced, as well. This example highlights the usefulness of our approach for uncovering the interaction structure between mutations in developing potent drugs.

The triple mutation M46I/I54V/V82A impairs the binding of indinavir. As mentioned before, these three mutations occur sequentially in specific orders. Because single mutation at 54 is not able to resist indinavir, the first mutation has to be at either 46 or 82 so that the mutant virus can have a better chance to survive the attack of indinavir. If the first mutation occurs at 46, the second mutation has to be at 82 because the double mutations at 46 and 54 cannot resist to indinavir, as well. If the first mutation is at 82, the subsequent mutation can be at either 46 or 54. We observed exactly these (and only these) three possible orders

Table 1. The binding free energies and the energy components calculated by MM/GBSA (kcal/mol)

No.	$\Delta E_{\text{vdw}}$	$\Delta E_{\text{ele}}$	$\Delta G_{\text{GB}}$	$\Delta G_{\text{SA}}$	$\Delta E_{\text{ele}} + \Delta G_{\text{GB}}$	$\Delta E_{\text{vdw}} + \Delta G_{\text{SA}}$	$\Delta G_{\text{cal}}$	$\Delta \Delta G_{\text{cal}}^*$
WT	$-80.00 \pm 0.16^{\dagger}$	$-25.59 \pm 0.35$	$33.97 \pm 0.35$	$-9.87 \pm 0.06$	$8.37 \pm 0.00$	$-89.87 \pm 0.22$	$-81.50 \pm 0.46$	0.00
Group 1								
M46I	$-77.43 \pm 1.85$	$-24.01 \pm 0.39$	$34.46 \pm 0.37$	$-10.32 \pm 0.64$	$10.44 \pm 0.76$	$-87.74 \pm 1.21$	$-77.30 \pm 0.45$	4.20
I54V	$-82.99 \pm 0.26$	$-25.38 \pm 0.21$	$36.13 \pm 0.16$	$-9.25 \pm 0.57$	$10.74 \pm 0.37$	$-92.24 \pm 0.83$	$-81.50 \pm 0.46$	0.00
V82A	$-75.98 \pm 1.27$	$-24.67 \pm 0.10$	$34.54 \pm 0.13$	$-9.56 \pm 0.00$	$9.87 \pm 0.23$	$-85.54 \pm 1.27$	$-75.67 \pm 1.50$	5.83
M46I/I54V	$-83.26 \pm 1.05$	$-22.01 \pm 1.23$	$34.19 \pm 1.44$	$-9.98 \pm 0.06$	$12.18 \pm 0.21$	$-93.24 \pm 1.11$	$-81.06 \pm 0.90$	0.44
M46I/V82A	$-70.84 \pm 2.86$	$-24.92 \pm 1.87$	$33.96 \pm 1.40$	$-9.67 \pm 1.43$	$9.04 \pm 0.47$	$-80.52 \pm 4.29$	$-71.48 \pm 4.76$	10.02
I54V/V82A	$-71.84 \pm 0.64$	$-19.62 \pm 0.92$	$31.08 \pm 1.06$	$-9.82 \pm 0.01$	$11.46 \pm 0.13$	$-81.66 \pm 0.63$	$-70.20 \pm 0.77$	11.30
M46I/I54V/V82A	$-79.58 \pm 0.91$	$-19.09 \pm 0.17$	$29.89 \pm 0.41$	$-8.92 \pm 0.59$	$10.79 \pm 0.24$	$-88.50 \pm 0.33$	$-77.71 \pm 0.08$	3.79
Group 2								
G73S	$-78.79 \pm 0.50$	$-24.76 \pm 0.51$	$33.26 \pm 0.14$	$-10.36 \pm 0.67$	$8.50 \pm 0.37$	$-89.15 \pm 0.17$	$-80.65 \pm 0.54$	0.85
L90M	$-80.56 \pm 0.32$	$-27.13 \pm 0.33$	$35.59 \pm 0.06$	$-9.78 \pm 0.06$	$8.46 \pm 0.39$	$-90.33 \pm 0.38$	$-81.87 \pm 0.00$	-0.37
G73S/L90M	$-81.31 \pm 0.37$	$-22.89 \pm 0.19$	$33.01 \pm 0.05$	$-9.80 \pm 0.00$	$10.12 \pm 0.24$	$-91.11 \pm 0.38$	$-80.99 \pm 0.14$	0.51

\* $\Delta \Delta G_{\text{cal}}$  is the difference between the binding free energy of the mutated complex and that of the wild-type.

<sup>†</sup>Standard deviations were estimated from two block average values.

(46 → 82 → 54, 82 → 46 → 54 and 82 → 54 → 46) in the Stanford HIVdb database. Our energy calculation and probabilistic modeling are all consistent with this sequential acquisition observation (Fig. 3).

Compared to the protease with a single mutation M46I, the additional mutation I54V makes more residues contributing favorably to the indinavir binding (nine vs. five). From Fig. 3B1 and C1 we can see that these nine favorable residues spread around the binding pocket, and thus enhance the binding of indinavir right in the pocket and block the function of protease. However, with V82A, the additional mutation I54V does not make indinavir interact more or less favorably with residues (seven vs. seven), which may superficially suggest that I54V would not affect the resistance caused by V82A. However, we can see from Fig. 3C2 that the seven favorable residues cluster tightly at one side of the binding pocket and the seven unfavorable ones at the other side. We speculate that such an uneven or asymmetric distribution of favorable/unfavorable residues may have pushed indinavir aside from blocking the binding pocket and thus reduced the potency of the drug.

The resistance caused by 70 and 90 cannot be explained by the binding free energy analysis that is consistent with observations made in the previous experiments (15, 16), suggesting that the group {73, 90} may follow a different resistant mechanism rather than impairing the binding affinity (SI Text).

**Two Drugs Attacking Reverse Transcriptase.** HIV-1 RT is a heterodimer consisting of p66 and p51 subunits. The p66 subunit is composed of all 560 amino acids of RT whereas p51 subunit is composed of the first 440 amino acids. RT is critical for RNA-dependent DNA polymerization and DNA-dependent DNA polymerization. We analyzed drug resistant mutation data of two drugs targeting RT: Zidovudine, a nucleoside analog reverse transcriptase inhibitor (NRTI), and Nevirapine, a non-nucleoside reverse transcriptase inhibitor (NNRTI).

Zidovudine is not designed to bind with RT and block the function of RT (unlike indinavir and nevirapine in the following) but rather to compete with natural dNTPs for incorporation into the newly synthesized DNA chains where it causes chain termination. Therefore, we cannot investigate its structural basis of resistant mutations by using MD simulations and free energy decompositions. To date, three biochemical mechanisms of NRTI drug resistance have been uncovered or proposed (3, 17). These different resistance mechanisms seem to correlate with different sets of mutations in RT (17), but further biochemical investigations are needed to confirm which mechanism corresponds to which independent mutation set (SI Text). Unlike NRTIs, NNRTIs bind to a hydrophobic pocket in RT close to the active site and their binding can block the catalytic activity of RT. The

RT mutations resistant to NNRTIs often occur in the hydrophobic binding pocket to deteriorate the inhibitors' binding.

We have analyzed two RT-related datasets in the Stanford HIVdb by using our statistical procedure: for zidovudine, 339 HIV-1 type B RT sequences from zidovudine-treated patients and 2187 sequences (HIV-1 type B) from untreated patients contain mutations at each position of the 190aa-long polypeptide sequences (from position 31 to 220 of RT); for nevirapine, 380 RT sequences from nevirapine-treated patients and 1622 RT sequences from untreated patients (both HIV-1 type C) correspond to the same 190aa-long region as in the zidovudine data. Any combination of mutations among these 190 positions may be related to the virus' drug resistance capability. Our goal is to find those positions that are either independently or interactively associated with each of the treatments.

**Interaction Patterns for Drug Resistance of Zidovudine.** Fig. S3A shows the interactively associated mutations the BVP method found, all of which are on the drug resistance mutation list. Table S2 shows all the mutation interaction patterns we found. The top three have a posterior probability >0.25. We have also checked the detailed configurations of the top interaction patterns with the phenotypic data [fold resistance from the Stanford HIVdb (Table S4)], which provide confirming evidence for the significant ones (after Bonferroni corrections).

As shown in Fig. S3B, the RMS procedure decomposed the set of interacting mutations {41, 67, 70, 210, 215, 219} into three independent groups: {41, 210, 215} for group one, {67, 219} for group two, and 70 for group three. For group one, it has been observed that mutations between M41L, L210W, and T215Y/F tend to occur together (3, 1819–20). We also inferred that L210W appears after T215Y/F, which is consistent with crystallographic studies. The aromatic side chain of Trp 210 can stabilize the interaction of Phe/Tyr215 with the dNTP-binding pocket (19). For group two, it has been observed earlier that these two mutations usually occur together (3). The finding that position 70 is independent of the others suggests that the R → K reversion of residue 70 may represent a compensatory mechanism allowing a functional rearrangement of the dNTP-binding pocket in the mutated RT (19).

**No Interactions Among Nevirapine-Resistant Mutations.** Our analyses of the nevirapine data suggested that the interactions among nevirapine-resistant mutations are very weak. As shown in Fig. S4A, the posterior probabilities for mutations 103, 181, 188, and 190 to interact are reasonably high under one prior distribution, whereas these probabilities diminished to near zero when another prior is used. Fig. S4B shows the total posterior probability for a mutation to resist to the drug, indicating that the results from using the two priors are consistent. Six mutations, 103, 106, 135, 181, 188, and





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tion. We model  $P_0(X_{ij})$  by a multinomial distribution (it is independent of  $Y_i$  because the variable is in group zero), denoted as multinom ( $\theta_j$ ), with  $\theta_j$  following a Dirichlet distribution a priori. Similarly, we model  $P_1(X_{ij}|Y_i)$  by multinom ( $\theta_{j,Y_i}$ ) with  $\theta_{j,Y_i}$  following a Dirichlet prior and model  $P_2(X_{i,G_2}|Y_i)$  by multinom ( $\Theta_{G_2,Y_i}$ ). For  $Y_i = 1$  (treated), the dimension of  $\Theta_{G_2,1}$  is equal to the cardinality of the support of  $X_{i,G_2}$  and  $\Theta_{G_2,1}$  follows a Dirichlet prior.

Ideally, all mutation positions among the untreated sequences ( $Y_i = 0$ ) should be mutually independent. Complications may arise, however. We thus introduce a model indicator variable  $J_{un}$  (same for all untreated individuals) so that the independence prior model  $\Theta_{G_2,0} = \prod_{j \in G_2} \theta_{j,0}$  holds only when  $J_{un} = 0$ , with  $\theta_{j,0}$  following a Dirichlet distribution;  $\Theta_{G_2,0}$  is fully saturated as  $\Theta_{G_2,1}$  when  $J_{un} = 1$ , following a full Dirichlet distribution. We observed that  $J_{un} = 0$  in most cases, that is, the mutations in  $G_2$  are mutually independent for untreated individuals. Conditional on  $I$  and  $J_{un}$ , we can integrate out all the multinomial parameters so as to have the posterior distribution of  $(I, J_{un})$ . A Markov chain Monte Carlo (MCMC) algorithm (9) can be designed to sample from this posterior distribution so as to infer which variables are associated with the treatment status. More details on BVP can be found in *SI Text*.

**Recursive Model Selection.** In the above BVP model, variables in  $G_2$  are not given any simplifying dependence structure, which in statistical term means that a “fully saturated” model was used. However, in practice, often a much more desirable and simpler model that takes advantage of conditional independence relationships among the variables can fit the data well. A possible approach is to infer a complete Bayesian network for all the variables in  $G_2$ . But this is computationally expensive and tends to over fit the limited amount of data. Our strategy is to first infer among two classes of cruder models, that is, the chain-dependence model and the  $V$ -dependence model, and then recursively apply this strategy until the data do not support more detailed models.

We say that a group of variables  $X_G$  follow a chain-dependence model if the index set  $G$  can be partitioned into three subgroups  $A$ ,  $B$ , and  $C$  such that  $X_A$  and  $X_C$  are independent given  $X_B$ , such as  $X_A \rightarrow X_B \rightarrow X_C$ . Only set  $C$  is

allowed to be empty, in which case this model degenerates to the saturated model. Under the chain-dependence model, we can decompose the joint distribution of  $X_G$  as:  $P(X_G) = P(X_A)P(X_B|X_A)P(X_C|X_B)$  (Fig. S5A). We say that  $X_G$  follow a  $V$ -dependence model if  $X_A$  and  $X_C$  are mutually independent, that is,  $P(X_G) = P(X_A)P(X_C)P(X_B|X_A, X_C)$ . In this case,  $X_B$  can be viewed as “children” of  $X_A$  and  $X_C$  (Fig. S5B). Although these models are not fully identifiable, RMS attempts to land in the best equivalent class of models.

We define a model indicator  $I_{CV}$ , which is equal to one for the chain-dependence model and zero for the  $V$ -dependence model. We let  $\Pi$  denote the set partition, that is, indicating which indices in  $G$  belong to which subset. In *SI Text*, we detailed the model likelihoods for the two competing models conditional on the partition  $\Pi$ , that is,  $P(D|\Pi, I_{CV} = 1)$  and  $P(D|\Pi, I_{CV} = 0)$ , where  $D$  denotes all the data. Assuming an equal prior probability for  $I_{CV}$ , we have that:

$$P(\Pi, I_{CV} | \text{Data}) \propto P(\text{Data} | \Pi, I_{CV}) P(\Pi) P(I_{CV}). \quad [1]$$

Here  $P(D|\Pi, I_{CV} = 1)$  and  $P(D|\Pi, I_{CV} = 0)$  can be computed, respectively, by using formulas (S5) and (S9) of *SI Text*. An MCMC algorithm is designed to simulate from (1) and to find the optimal model type and variable partition. The procedure is applied recursively until only single-variable nodes are available. We applied RMS to both treated data and untreated data separately. Fig. 2 illustrates the structure we found in the treated data (Fig. S7 shows the details of recursion). In contrast, we could not find an unambiguous structure in the untreated data.

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